

REMARKS

Responsive to the examiner's office action dated January 4, 2002, and further in view of the interview carried out on June 4, 2002, applicants respectfully request entry of the above amendments. The specification supports these amendments, and specifically, for claims 12-19 support is found at p.4:15-18, for claims 20 and 22 at p.10:10-46, and originally filed claim 2 supports claims 21 and 23.

Greener et al. and Wilks et al., on which rejection has been based, do not teach all elements of the present claims. Accordingly, applicants request withdrawal of the rejections of record under 35 USC §102(b) and §103(a).

In view of the foregoing amendments and remarks, applicants consider that the rejections of record have been obviated and respectfully solicit passage of the application to issue.

**Please find attached a check for \$400.00 for a 2 month extension of time.**

Please charge any shortage in fees due in connection with the filing of this paper, including Extension of Time fees to Deposit Account No. 11-0345. Please credit any excess fees to such deposit account.

BORNSCHEUER et al. Ser. No. 09/161,680

Respectfully submitted,  
KEIL & WEINKAUF

A handwritten signature in black ink, appearing to read 'David C. Liechty', with a long horizontal flourish extending to the right.

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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**IN THE CLAIMS**

Please cancel claims 1-2, 4-7 and 11.

Please enter new claims 12 to 23, which read as follows:

12. (newly added) A method for altering the substrate specificity of an enzyme to a substrate from a substrate specificity where catalysis does not occur to a substrate specificity where catalysis does occur, comprising the steps of:
- a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof,
  - b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,
  - c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,
  - d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize altered substrate specificity of the enzyme, with or without other indicator substances,
  - e) selecting the microorganisms which show an alteration in the substrate specificity, said microorganisms in steps b), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts,
- wherein the enzyme is selected from the group consisting of lipases, amidases,

nitrilases, ether hydrolases, peroxidases, glycosidases and phytases.

13. (newly added) The method of claim 12, wherein the enzyme is a lipase.
14. (newly added) The method of claim 12, wherein the enzyme is an amidase.
15. (newly added) The method of claim 12, wherein the enzyme is a nitrilase.
16. (newly added) The method of claim 12, wherein the enzyme is an ether hydrolase.
17. (newly added) The method of claim 12, wherein the enzyme is a peroxidase.
18. (newly added) The method of claim 12, wherein the enzyme is a glycosidase.
19. (newly added) The method of claim 12, wherein the enzyme is a phytase.
20. (newly added) The method of claim 13, wherein the lipase is selected from the group of lipases consisting of *Pseudomonas cepacia* lipase PS, *Pseudomonas cepacia* lipase AH, acylase, *Rhizopus delamar* lipase, *Rhizopus javanicus* lipase, *Candida rugosa* lipase, *Mucor javanicus* lipase, *Penicillium roquefortii* lipase, *Penicillium cyclopium* lipase, *Chromobacterium viscosum* lipase, *Rhizomucor*

*miehei* lipase, *Humicola lanuginosa* lipase, *Candida antarctica* lipase B and *Candida antarctica* lipase A.

21. (newly added) The method of claim 12, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.
22. (newly added) A method for altering the substrate specificity of an enzyme to a substrate from a substrate specificity where catalysis does not occur to a substrate specificity where catalysis does occur, comprising the steps of:
  - a) introducing a DNA sequence coding for the enzyme into the *Escherichia coli* strain XL1-Red or into a functional derivative thereof,
  - b) incubating the transformed *Escherichia coli* strain XL1-Red or its functional derivative to generate mutations in the DNA sequence,
  - c) transferring the mutated DNA sequence from the transformed *Escherichia coli* strain XL1-Red or its functional derivative to a microorganism which has no impeding enzyme activity,
  - d) incubating this microorganism to detect the enzyme activity in at least one selection medium which comprises at least one enzyme substrate to recognize altered substrate specificity of the enzyme, with or without other indicator substances,

- e) selecting the microorganisms which show an alteration in the substrate specificity, said microorganisms in steps b), d) and e) being a member selected from the group consisting of bacteria, fungi and yeasts, wherein the enzyme is an esterase selected from the group consisting of *Pseudomonas fluorescens* esterase, pig liver esterase and *Thermoanaerobium brockii* esterase.
23. (newly added) The method of claim 22, wherein steps (a) to (e) are performed several times in sequence by reisolating and retransforming the DNA sequence from the microorganisms selected in step (e) to the strain *Escherichia coli* XL-1 Red or its functional derivative.